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We report herein the cloning, expression, and seroreactivity of three *Leishmania tropica* antigens. By ELISA analysis, the majority of confirmed and suspected viscerotropic leishmaniasis patients had significantly higher levels of specific antibody against two of the three recombinant antigens and the repeat portion of one of the antigens, named Lt-1r, when compared to sera from normal controls. The molecular characterization of these two antigens demonstrated that these are adjacent portions of the same gene and that the repeat portion contains one of the immunodominant epitopes. In addition, this gene was shown to be conserved in all strains of *L. tropica* parasites recovered from Gulf War participants. The gene does not appear to be highly conserved among all species of *Leismania*. Collectively, the data indicate the potential for these antigens to assist in the diagnosis of individuals with viscerotropic leishmaniasis.

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INTRODUCTION

Infection by the parasite *Leishmania* can result in a broad spectrum of pathological outcomes in the human host, ranging from simple self-healing cutaneous lesions to visceral leishmaniasis (VL), commonly referred to as kala-azar, with symptoms including fever, emaciation, hypergammaglobulinemia, hepatosplenomegaly, and pancytopenia. The differing pathologies are correlated with infection by differing species, particularly in the African and Eurasian species. *Leishmania donovani*, *L. chagasi*, and *L. infantum* usually cause visceral leishmaniasis and *L. major* and *L. tropica* generally cause cutaneous leishmaniasis. Exceptional cases have been previously described, such as visceral outcomes in individuals infected with *L. tropica* (1, 2).

More recently, exposure of a yet unknown number of individuals during the Gulf War to *L. tropica* has resulted in a variant form of visceral disease in several individuals with confirmed infection with this species of *Leishmania* (3, 4). Additional confirmed cases continue to arise at this time (Dr. A. Magill-personal communication). This form of disease is associated with variable symptoms including those seen in patients with mild forms of kala-azar. This form of visceral disease, referred to as viscerotropic leishmaniasis (VTL), differs from classical visceral disease in the variable pathology observed, with several patients lacking both fever and hepatosplenomegaly (4). In addition, serum anti-leishmanial antibody titers are variable, but generally much lower than those observed in patients with classical visceral leishmaniasis.

Diagnosis of classical visceral leishmaniasis has utilized the high antibody response to parasite antigens in tests involving reactivity to promastigotes or to recombinant antigens. Confirmation is achieved by the isolation of live parasites from spleen, liver, bone marrow, or lymph nodes. Diagnosis using reactivity to parasite promastigotes in patients with VTL leishmaniasis is not likely to be successful due to their low overall reactivity.

An alternative strategy we have employed is based on the presumption that these patients are strongly reactive against one or more immunodominant antigens, but that these antigens are either expressed by the parasite at very low levels or predominantly in the amastigote stage. Because it is impractical to obtain *L. tropica* amastigotes, molecular cloning is the best approach for obtaining such antigens. We report here the isolation, expression, and seroreactivity of three immunodominant antigens recovered through expression screening of *L. tropica* genomic library. In general, sera from viscerotropic leishmaniasis patients contain significantly higher levels of specific antibody directed against these two antigens when compared to sera from normal controls.

MATERIALS AND METHODS

Parasites. *L. tropica* isolate (MHOM/SA/91/WR1063SS), (MCAN/SA/91/WR1091SS), (MHOM/SA/91/WR1092SS), (MHOM/IQ/91/WR1095SS), and (MHOM/SA/92/WR2044SS), *Leishmania amazonensis* (IFLA/BR/67/PH8), *Leishmania braziliensis* (MHOM/BR/75/M2903), *L. chagasi* (MHOM/BR/82/BA-2,C1), *L. donovani* (MHOM/Et/67/HU3), *Leishmania guyanensis* (MHOM/BR/75/M4147), *L. infantum* (IPT-1), *L. major* (LTM p-2), *L. major* (Friedlander), and *Trypanosoma cruzi* (MHOM/CH/00/Tulahuen C2) were used. *Leishmania* promastigotes and *T. cruzi* epimastigotes were cultured in axenic media.

Sera. Groups of sera from viscerotropic leishmaniasis (VTL) patients, gulf war syndrome (GWS) patients, and Army normals were received from Drs. M. Grogl and A. Magill. Normal sera were received from Red Cross, Portland, OR. In addition, sera from visceral leishmaniasis patients, confirmed by parasite detection, were obtained from Brazil and Sudan, by Drs. R. Badaro and H. Ghalib, respectively.

Isolation of Lt-1, Lt-2, and Lt-3. *L. tropica* (MHOM/SA/91/WR1063C) genomic DNA was isolated and sheared by passage through a 30-gauge needle to a size range of 2-6 kilobase. The library was constructed in Lambda ZapII (Stratagene) using EcoR1 adaptors. Expression screening was performed using a pool of pre-adsorbed patient sera (5).

Expression of recombinant *L. tropica* antigens. Induced bacterial pellets were lysed in buffer (LB, 50 mM TRIS-HCL, pH 8.0, 100 mM NaCl, 10 mM EDTA) by lysosyme and sonication. rLt-1, rLt-1r and rLt-2 were recovered from the inclusion bodies. rLt-1 and rLt-2 were solubilized in 8M urea and rLt-1r solubilized in 4M urea. rLt-3 was recovered in the soluble fraction. The recombinant proteins rLt-1, rLt-2, and rLt-3 were purified from the 25-40% ammonium sulfate fraction and rLt-1r purified from the 10-25% ammonium sulfate fraction by preparative gel separation in 10% SDS-PAGE. Recombinant proteins were eluted from the gels and dialyzed in PBS, the concentration measured by the Pierce BCA assay, and purity assessed by SDS-PAGE followed by Coomassie blue staining.

Molecular analysis of Lt-1, Lt-2, and Lt-3. Exonuclease III digestion was used to create overlapping deletions of the clones. Single strand template was prepared and sequenced (Applied Biosystems Automated Sequencer model 373A). The entire coding strand sequence of all three clones was determined excepting the domains containing repeated sequence, which were only partially sequenced.

Genomic DNA was restricted with a variety of enzymes, separated by agarose gel electrophoresis, and blotted on Nytran (S.& S.). The *L. tropica* inserts were labeled with ^{32}P dCTP by random oligonucleotide primers (Boehringer Mannheim) and used as probes after purification by GS-50 sephadex columns. Hybridizations were performed at 65° in .2 M Na H_2PO_4 /3.6 M NaCl /.2 M EDTA overnight and washed to a stringency of .075 M NaCl/.0075 M sodium citrate, pH 7.0/.5% SDS at 65°.

ELISA. Microassay plates (Falcon 3915) were coated with recombinant antigen or parasite lysate in coating buffer (15 mM Na_2HCO_3 /28 mM NaH_2CO_3, pH 9.6) and incubated overnight at 4° C or 1 hour at 37°. Plates were blocked with phosphate-buffered saline (PBS) with 1% Tween-20, 1 hour and washed 5X with PBS, .1% Tween-20 (PBS-T). Sera was added (50 ul of 1:50 dilution), and incubated 30 minutes at room temperature on a shaker. Plates were washed 5X with PBS-T. Bound antibody was detected with protein A-conjugated horseradish peroxidase (Zymed) as previously described (6). Absorbance values reported relative to the mean of 5 control sera. Two sample t-test was used in statistical analysis.

Immunoblot analysis. *L tropica* parasite crude lysate, purified rLt-1, and purified rLt-1r were subjected to SDS-PAGE in a 12% gel and transferred to nitrocellulose (15 minutes at 50V, 1 hour at 100V). Filters were blocked with PBS containing 5% nonfat dry milk at 4° overnight, washed 3X in PBS-T, and incubated 1 hour in sera (diluted 1:50 in PBS-T) on rocker at room temperature. Filters were washed 3X with PBS-T and bound antibody detected with 10^5 cpm/ml ^{125}I-labeled protein A followed by autoradiography.

RESULTS

Isolation of recombinant *L. tropica* genes. Approximately 43,000 recombinant phage were screened using a pool of pre-adsorbed patient sera, resulting in the identification of three immunoreactive clones ranging from 1.4-3.3 kilobase in size encoding recombinant antigens of 75, 70, and 120 kilodaltons, referred to respectively as Lt-1, Lt-2 , and Lt-3. Recombinant antigens were expressed and purified. The induction and purified recombinant protein Lt-1 is shown in Figure 1 (lanes 1-4).

Southern analysis of *L. tropica* genes. Genomic DNA from a number of *Leishmania* species including *L. tropica* were analyzed by Southern blots using the three clones separately as probes. Using Lt-2 as a probe, various digests of *L. tropica* DNA indicate that a low copy number of this gene is likely (Fig. 2B). The comparison of hybridization intensities to PstI digest of numerous species yielded a surprising result. Strong hybridization was observed with *L. tropica* and members of the *L. donovani* complex including *L. donovani*, *L. chagasi*, and *L. infantum*, but none was observed with *L. major*, a species considered to be closely related to *L. tropica* (Fig. 2B). In addition, some weak hybridization was observed with *L. amazonensis*, but none was seen with *L. braziliensis*, *L. guyanensis*, or *T. cruzi*. A similar, overlapping pattern was observed when probing the same blot, after stripping, with the Lt-1 probe (Fig. 2A), indicating that these two probes contain sequences near or overlapping one another. Very weak hybridization with *L. major* was also observed. Probing the blot with the Lt-3 clone yielded a more conserved pattern, with hybridization observed in all *Leishmania* species tested (data not shown).

Genomic DNA from five isolates of *L. tropica*, four from VTL patients and one from a dog with cutaneous leishmaniasis (3), and two isolates of *L. major* were digested with PstI and XhoI and analyzed by Southern blot, probing with the Lt-2 clone. The five different *L. tropica* isolates yielded similar intensities and restriction patterns, with only a single restriction fragment length polymorphism among the two digests of the five isolates (data not shown). No hybridization was observed in the PstI digests of the two *L. major* isolates, and only very weak hybridization was seen in the XhoI digests. Collectively, the data indicate strong similarity in this region among the five *L. tropica* isolates, and a striking divergence compared to *L. major* isolates. The similarity observed in this region among the different *L. tropica* isolates is relevant in consideration of its possible use as a diagnostic. A useful diagnostic must be genetically conserved among the different isolates involved, as Lt-1 and Lt-2 appear to be. Conversely, it is likely that the most useful diagnostic antigens will not be well conserved outside the species or genus of interest, as is the case of Lt-1 and Lt-2.

DNA sequencing of Lt-1, Lt-2, and Lt-3. Sequence analysis of the three *L. tropica* clones was performed. All three clones were found to contain a substantial portion of repeated sequence (Fig. 3). The repeat in Lt-1 was located in the 5' portion of the clone, consisting of approximately 12 copies of a 99 base pair sequence. The repeat in Lt-2 was in the 3' half of the clone and consists of a mixture of 60 and 99 base pair repeats. The sequence of the 99 base pair repeat contains the 60 base pair repeat and is identical, excepting occasional degeneracies, to the 99 base pair repeat in Lt-1. This is consistent with the Southern analysis data, which argued for adjacent or overlapping positions for Lt-1 and Lt-2. The sequencing data suggest that Lt-1 and Lt-2 are different portions of the same gene, with Lt-2 just upstream to Lt-1, with possibly a small overlap. The sequence analysis of Lt-3 revealed a substantial block of 24 base pair repeats toward the 5' end of the clone (Fig. 3). The nested deletion set of Lt-1 formed for sequencing included a deletion clone containing the 5' portion of the repeat sequence. This clone, referred to as Lt-1r (Fig. 3), consisting of slightly less than two repeats, was also expressed as a fusion protein and purified (Figure 1, lanes 5-8).

Homology searches were performed on the Lt-1, Lt-2, and Lt-3 sequences. No significant homology was found for Lt-1 or Lt-2 in the databases. Significant homology was found between Lt-3 and a *T. brucei* membrane associated protein, Tb-292. There was 40% identity between the two genes in the non-repeat region, and approximately 67.5% identity in the repeat region, which consisted of eight amino acid repeats in both genes.

Patient sera reactivity with *L. tropica* recombinant antigens. Evaluation of the recombinant antigens by ELISA was performed using sera from the following groups: VTL patients confirmed through parasite isolation (VTL-C), VTL patients suspected to have *L. tropica* infection based on PCR or mAb (VTL-S), Gulf War Syndrome patients (GWS), and two different

sets of normals (Army normals and Red Cross normals). Reactivity of the set of Army normals to rLt-1, rLt-1r, and rLt-2 was found to be consistently higher than the reactivity by the Red Cross normals. The relatively small sample size (n=12) was partially responsible for the differences observed. Indeed, over one-third of the difference between the two normal means for rLt-1, rLt-1r, and rLt-2 reactivity can be explained by a single high Army normal. If this explanation is correct, then the Red Cross normal group (n=32) is a more accurate reflection of the normal population. Alternatively, the difference could possibly be due to inherent differences in the two normal groups being used. One possibility would be a more recent vaccination schedule or history of exposure to *Leishmania* in the Army normal group due to military regulations and that cross reactive epitopes exist for the rLt-1 and rLt-1r antigens.

Initial ELISA evaluation of rLt-1 using sera from VL patients and VTL-C patients demonstrated that this antigen was immunodominant. Similar tests revealed that rLt-3 reacted strongly with VL sera, but reactivity with VTL-C sera was not significantly greater than that seen with normals (p=.38). Further ELISA analysis was confined to rLt-1, rLt-1r, and rLt-2.

Mean reactivity to *L. tropica* parasite lysate (Fig. 4) is not significantly increased in either the VTL-C group or the VTL-S group compared to normals. Mean reactivity to rLt-1(Fig. 4) is significantly higher in the VTL-C group (p=.05) but not the VTL-S group (p=.15) compared to Red Cross normals. A significant increase in mean reactivity to rLt-1r (Fig. 5) is observed in both the VTL-C group (p<.001) and the VTL-S group (p=.03) relative to Red Cross normals. Mean reactivity to rLt-2 (Fig. 6) is also significantly higher in the VTL-C group (p=.03) compared to Red Cross normal reactivity.

Immunoblot analysis was performed with parasite lysate, rLt-1, and rLt-1r, using sera from one confirmed VTL patient, one suspected VTL patient, and a pool of three normal sera (Fig. 7). Increased reactivity to both recombinant antigens was observed in the confirmed and suspected patients compared to the normals. Reactivity to parasite promastigote lysate was variable. These results indicate that the Western blot with recombinant *L. tropica* antigens may be a useful confirmatory test for *L. tropica* infection.

CONCLUSIONS

Serological screening of a recombinant *L. tropica* genomic DNA library has resulted in the isolation of three clones which encode immunodominant antigens. ELISA analysis was performed using the *L. tropica* antigens rLt-1, rLt-1r, and rLt-2 in a comparative test to a variety of other recombinant *Leishmania* antigens including *L. chagasi* hsp70, *L. braziliensis* hsp83/90, *L. braziliensis* eIF4A, *L. chagasi* rK-39, *L. braziliensis* mhsp70, *L. braziliensis* P0, and two *L. tropica* antigens isolated by expression screening with *L. major* sera. In these comparisons, rLt-1, rLt-1r, and rLt-2 were more reactive with the confirmed VTL patient sera than any of the other antigens tested. The results are consistent with the premise that these are immunodominant antigens and are the best serodiagnostic candidates isolated thus far. rLt-1 was highly reactive with sera from patients with acute visceral leishmaniasis. The high reactivity observed with this sera indicates that a strong humoral response can be mounted against this antigen upon infection with leishmanial parasites of the *L. donovani* complex.

The molecular characterization of the *L. tropica* clones revealed extensive repeat structure in all three clones. Repeat containing antigens that are immunodominant in both *Leishmania* (7) and *T. cruzi* (8) have been previously isolated by us. To address this possibility in rLt-1, a portion of the repeat was expressed separately as rLt-1r. The results indicate that the repeat does contain an immunodominant epitope.

The data presented here argue that the patients are not unresponsive to leishmanial antigens, but instead are responding to immunodominant antigens. These serological responses

are, however, limited compared to those seen in individuals with classical visceral disease where serological diagnosis is more straightforward. It seems likely that to generate sufficient signal relative to background reactivity, two things will be needed. First, the system will require multiple immunodominant antigens. Secondly, more detailed epitope mapping will be essential to separate the desired epitope(s) from others that convey unwanted non-specific reactivity.

It is encouraging that reactivity of three sera (Elliot, Brandes, Bachman) received, August 23, 1993, with the recombinant antigens rLt-1 and rLt-1r suggested infection with *L. tropica.* Subsequently, evidence of infection was demonstrated, with parasites cultured from Bachman, and evidence by PCR that Elliot and Brandes were infected. This demonstrates the possible usefulness of the recombinant antigens in assisting diagnosis and in accurately predicting VTL.

The greatest limitation of the *L. tropica* recombinant antigens tested is the percentage of normals that have seroreactivity in patient range. These false positives can potentially be reduced by the localization of immunodominant epitopes. This has been accomplished to some degree by the isolation of rLt-1r, containing just under two copies of the repeat present in rLt-1r. However, using this single epitope, some of the VTL patients may be missed, such as low reactors like Molina, Waddel, Neurone, and Vaughan. Nonetheless, it is apparent that these antigens contain strong serological epitopes, and that more precise mapping of these epitopes with the use of peptides should effectively eliminate or reduce serological responses in control sera, as we have previously reported (7, 8). Alternatively, it is possible that a different assay system may more clearly distinguish infected from uninfected individuals. The specificity of reactivity to the important epitope(s) in the recombinant antigen rLt-1r can possibly be improved by the construction of overlapping peptides along the 59 amino acids of the *L. tropica* insert. The recombinant antigen rLt-1r is approximately 18 kilodalton in size, which means that approximately 11 kilodaltons is composed of 5' and 3' vector sequences. It is quite possible that some of the reactivity observed in normals is to epitopes in these regions, which will be eliminated by the utilization of peptides. These peptides are currently being constructed. The mean reactivity found in the Red Cross normal group (n=32) was at an acceptably low level that allowed distinction of the majority of confirmed and suspected *L. tropica* infected individuals. However, the mean reactivity of the Army "normals" was elevated.

An additional handicap to providing an effective diagnostic has been the limited number of patients, the limited amount of sera available from these patients, and the limitation in the number of collection times. It would have been very useful to have had sera taken at various time points, such as pre-infection, pre-treatment (after initial presentation), and several time points post-treatment to be able to determine seroreactivity during the course of the disease. Our assays were performed using small quantities of sera taken at single time points, not necessarily at similar points in the disease progression, among the infected individuals. The majority of these difficulties are the result of the disease itself, since onset of disease consisted of variable symptoms and occurred after variable and extended latency periods with many of the patient sera we have included in this study coming from individuals not diagnosed until well after the isolation of the recombinant antigens used.

The utility of rLt-1 extends beyond serology. Recently, in collaboration with Dr. A. Magill, we have found that this antigen elicits significant levels of interferon-gamma from peripheral blood mononuclear cells (PBMC) from both leishmaniasis confirmed and suspect ODS patients. In contrast, PBMC from normal individuals did not make detectable interferon-gamma in response to rLt-1. Thus, it appears that interferon production in response to rLt-1 may be more specific for distinguishing ODS patients from non-leishmaniasis controls. Experiments are in progress to confirm and expand these observations.

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Table 1. ELISA values of ODS patient and control sera using *L. tropica* lysate or recombinant *L. tropica* antigens.

Serum sample	Classification	*L.tropica* lysate	rLt-1	rLt-1r	rLt-2
Malloy	VTL-C	.084	.283	.713	.298
Waddel	VTL-C	.161	.155	.262	.056
Molina	VTL-C	.130	.141	.275	.084
Bachman	VTL-C	.000	.258	.202	1.278
Boone	VTL-C	.074	.091	.467	.254
Ford	VTL-C	.116	.084	.462	.152
Patrick	VTL-C	.478	.145	.552	.166
Mean (SE)		.149 (.058)	.165 (.029)	.419 (.069)	.327 (.162)
Elliot	VTL-S	.000	.302	.530	.241
Brandes	VTL-S	.155	.233	.506	.111
Nerone	VTL-S	.000	.050	.116	.043
Vaughan	VTL-S	.022	.078	.214	.148
Mean (SE)		.044 (.037)	.166 (.061)	.342 (.104)	.136 (.041)
Sergott	GWS	.071	.070	.230	.060
Lyon	GWS	.000	.441	.252	.308
Kish	GWS	.160	.102	.191	.104
Mean (SE)		.077 (.046)	.204 (.119)	.224 (.018)	.157 (.076)
Cohen	ARMY-N	.005	.111	.372	.173
Kishpaugh	ARMY-N	.035	.229	.226	.333
Scheumann	ARMY-N	.041	.158	1.052	.382
DeArmas	ARMY-N	.212	.815	.228	.860
Ware	ARMY-N	.241	.232	.155	.179
Wood	ARMY-N	.067	.028	.402	.147
Boor	ARMY-N	.192	.153	.410	.174
Russell	ARMY-N	.078	.013	.144	.131
Sanchez	ARMY-N	.129	.187	.433	.093
Sperry	ARMY-N	.056	.274	.101	.153
Johnson	ARMY-N	.211	.221	.343	.361
Figueroa	ARMY-N	.100	.024	.417	.137
Mean (SE) n=12 normals		.114 (.023)	.204 (.061) .148(.028) n=11	.357(.072) .290 (.040) n=11	.260 (.061) .205(.031) n=11
9/10/93-4	RC-N	.000	.010	.034	.010
9/9/93-1	RC-N	.010	.010	.027	.011
8/31/93-2	RC-N	.057	.029	.068	.032
11/17/93-4	RC-N	.074	.026	.121	.048
9/9/93-2	RC-N	.095	.042	.057	.039
7/1/93-1	RC-N	.244	.571	.075	.227
4/15/93-3	RC-N	.022	.019	.084	.040
6/8/93-1	RC-N	.118	.059	.368	.116
12/22/92-4	RC-N	.029	.043	.176	.046
6/23/93-1	RC-N	.061	.036	.193	.099
615/93-2	RC-N	.093	.080	.510	.140
6/8/93-2	RC-N	.154	.028	.140	.060
5/26/93-3	RC-N	.056	.024	.179	.061
7/1/93-4	RC-N	.184	.066	.439	.125
6/15/93-3	RC-N	.154	.061	.286	.135
11/17/93-2	RC-N	.202	.075	.104	.779
11/17/93-1	RC-N	.110	.083	.061	.278
5/26/93-1	RC-N	.185	.022	.124	.177
5/26/93-4	RC-N	.086	.081	.339	.091
6/4/93	RC-N	.047	.024	.029	.036
11/17/93-3	RC-N	.172	.254	.088	.279
9/10/93-2	RC-N	.000	.030	.104	.053
9/10/93-3	RC-N	.000	.015	.100	.039
7/1/93-3	RC-N	.109	.103	.269	.077
7/1/93-2	RC-N	.109	.089	.330	.153
6/8/93-4	RC-N	.117	.039	.142	.135
6/16/93-4	RC-N	.053	.258	.274	.192
6/8/93-3	RC-N	.102	.068	.411	.161
6/16/93-3	RC-N	.126	.050	.101	.019
3/31/93-4	RC-N	.076	.068	.268	.073
6/15/93-1	RC-N	.134	.102	.174	.028
4/15/93-2	RC-N	.028	.056	.152	.084
Mean (SE) n=32 normals		.094 (.011)	.082 (.019)	.182 (.023)	.120 (.025)

FIGURE LEGENDS

Figure 1. Expression and purification of rLt-1 and rLt-1r. Coomassie Blue stained gel showing molecular weight markers in kilodaltons (lanes 1, 5), uninduced rLt-1 and rLt-1r (lanes 2, 6), induced rLt-1 and rLt-1r (lanes 3, 7), and purified rLt-1 and rLt-1r protein (lanes 4, 8).

Figure 2. Southern blot analysis of Lt-1 and Lt-2 sequences. Genomic DNA (2.5 ug/lane) of *L. tropica* was digested with *EcoR* I (lane 1), *Xba* I (lane 2), *Xho* I (lane 3), *BamH* I (lane 4), *Hind* III (lane 5), *Pst* I (lane 6). *L. major* (lane 7), *L. donovani* (lane 8), *L. infantum* (lane 9), *L. chagasi* (lane 10), *L. amazonensis* (lane 11), *L. braziliensis* (lane 12), *L. guyanensis* (lane 13), and *T. cruzi* (lane 14) were digested with *Pst* I. Blots probed with Lt-1 sequence (A) or Lt-2 sequence (B).

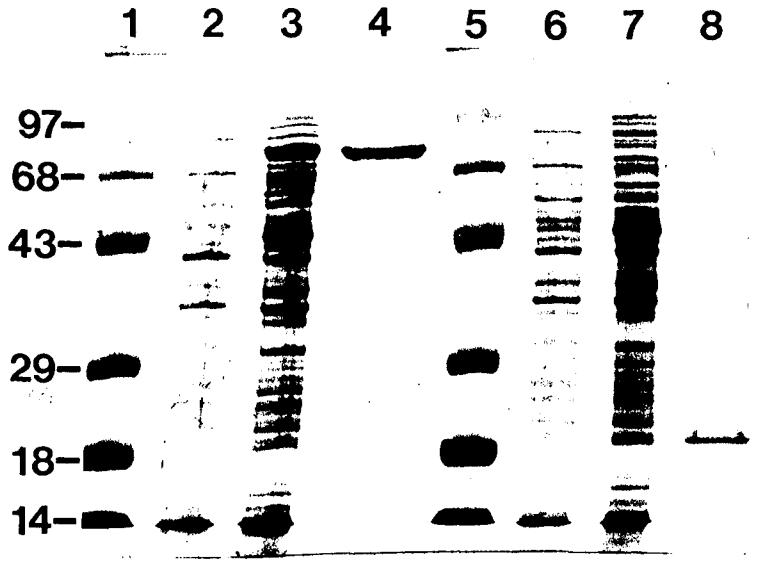
Figure 3. Schematic of *L. tropica* inserts cloned through expression screening. Shaded regions represent repeated DNA sequence and unshaded regions represent unique DNA. Sequence 3' of open reading frame in Lt-1 shown by solid line.

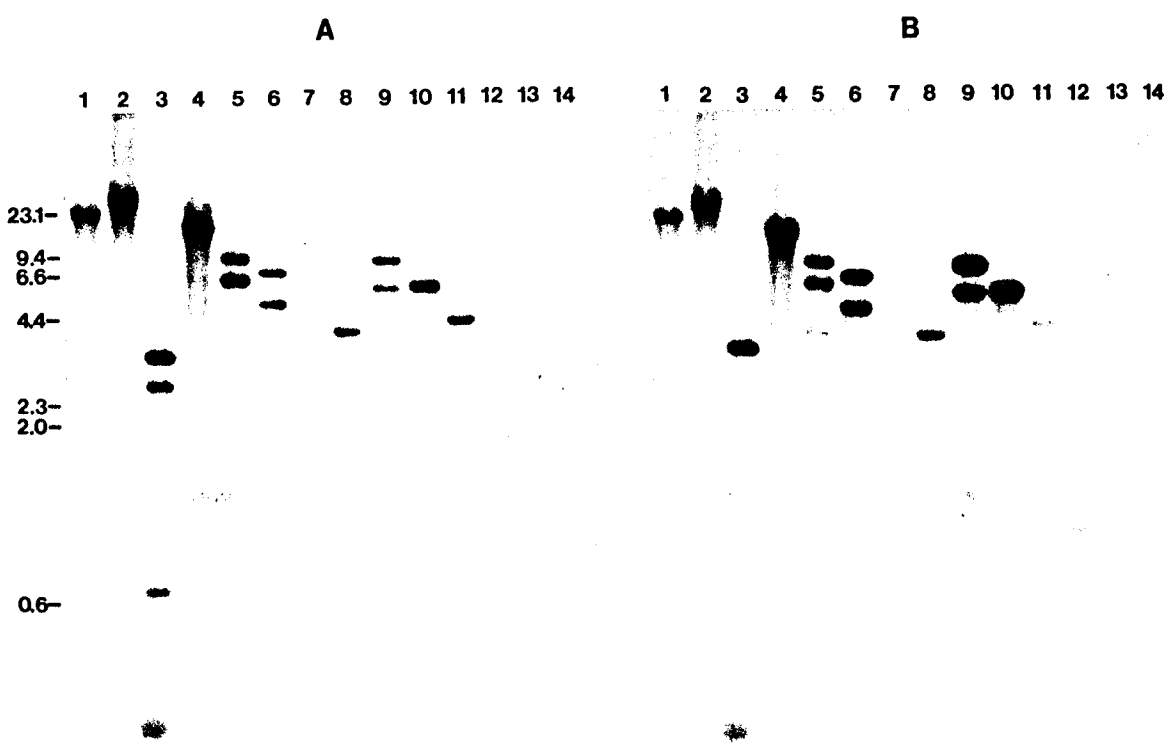
Figure 4. ELISA evaluation of VTL sera using *L. tropica* lysate and rLt-1. Absorbance values (mean $\pm$ SE) shown for confirmed viscerotropic patient (VTL-C, n=7), suspected viscerotropic patient (VTL-S, n=4), gulf war syndrome (GWS, n=3), Red Cross normal (RC-N, n=32), and Army normal (A-N, n=11, outlier value excluded) sera used at 1:50 dilution on either 1 ug *L. tropica* lysate or 50 ng rLt-1.

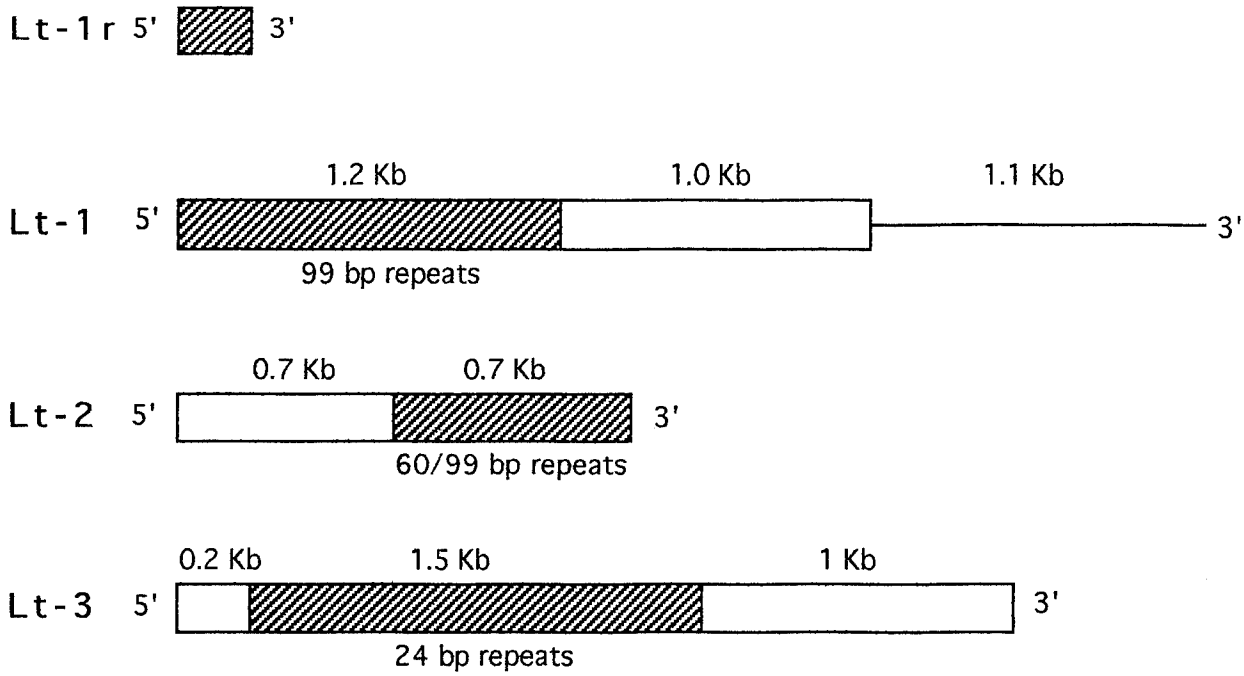
Figure 5. ELISA evaluation of VTL sera using *L. tropica* lysate and rLt-1r. Absorbance values (mean $\pm$ SE) shown for confirmed viscerotropic patient (VTL-C, n=7), suspected viscerotropic patient (VTL-S, n=4), gulf war syndrome (GWS, n=3), Red Cross normal (RC-N, n=32), and army normal (A-N, n=11, outlier value excluded) sera used at 1:50 dilution on either 1 ug *L. tropica* lysate or 25 ng rLt-1r.

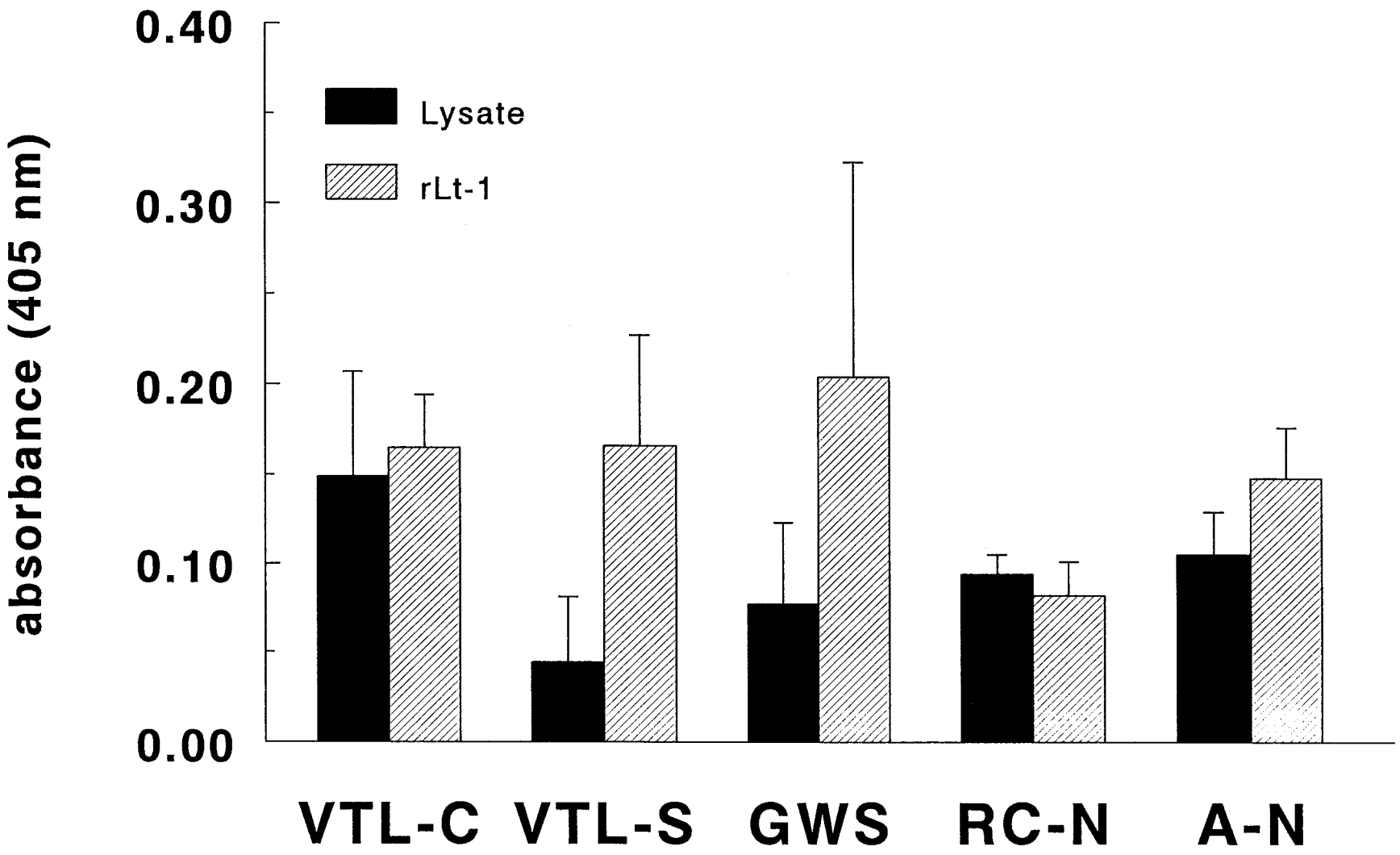
Figure 6. ELISA evaluation of VTL sera using *L. tropica* lysate and rLt-2. Absorbance values (mean $\pm$ SE) shown for confirmed viscerotropic patient (VTL-C, n=7), suspected viscerotropic patient (VTL-S, n=4), gulf war syndrome (GWS, n=3), Red Cross normal (RC-N, n=32), and army normal (A-N, n=11, outlier value excluded) sera used at 1:50 dilution on either 1 ug *L. tropica* lysate or 10 ng rLt-2.

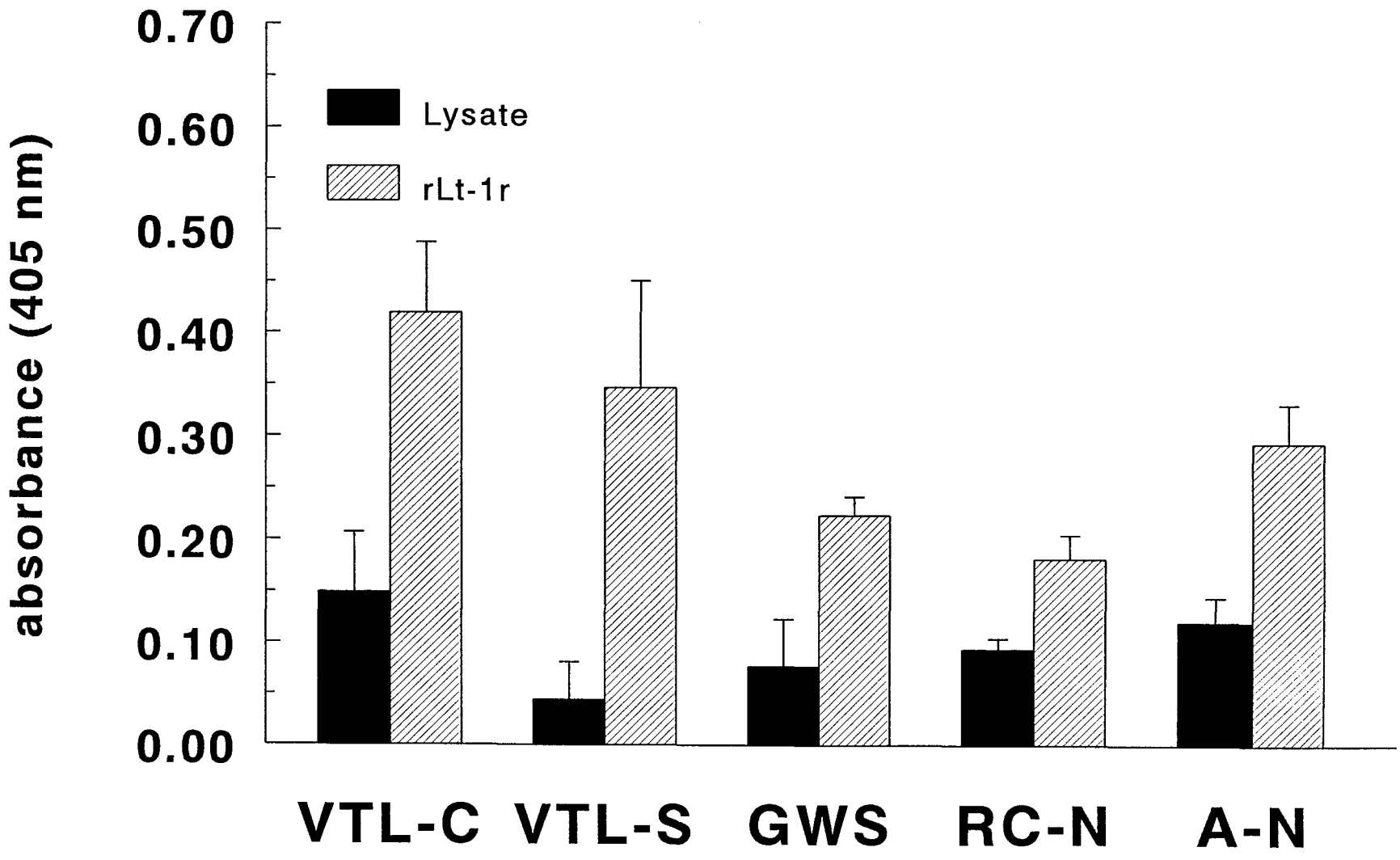
Figure 7. Immunoblot analysis of *L. tropica* lysate, rLt-1, and rLt-1r. *L. tropica* lysate (15 ug), rLt-1 (2 ug), and rLt-1r (2 ug) on blots were either stained with Coomassie blue (lanes 2-4) or probed with VTL-C sera (Bachman, lanes 5-7), VTL-S sera (Elliot, lanes 8-10), or pool of normal sera (Red Cross, n=3, lanes 11-13). Molecular markers shown in lane 1.

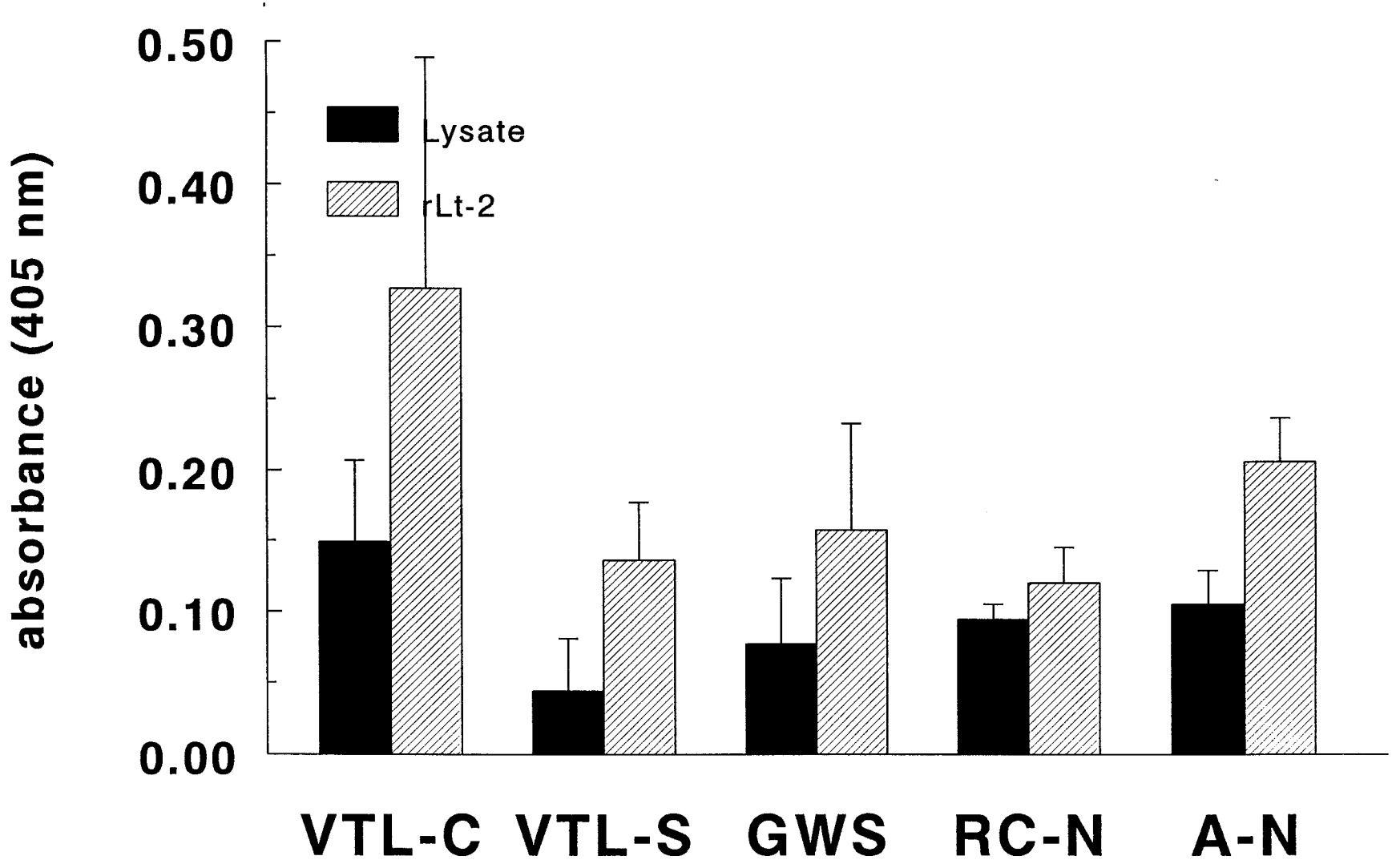

1
2
3
4
5
6
7
8
97–
68–
43–
29–
18–
14–

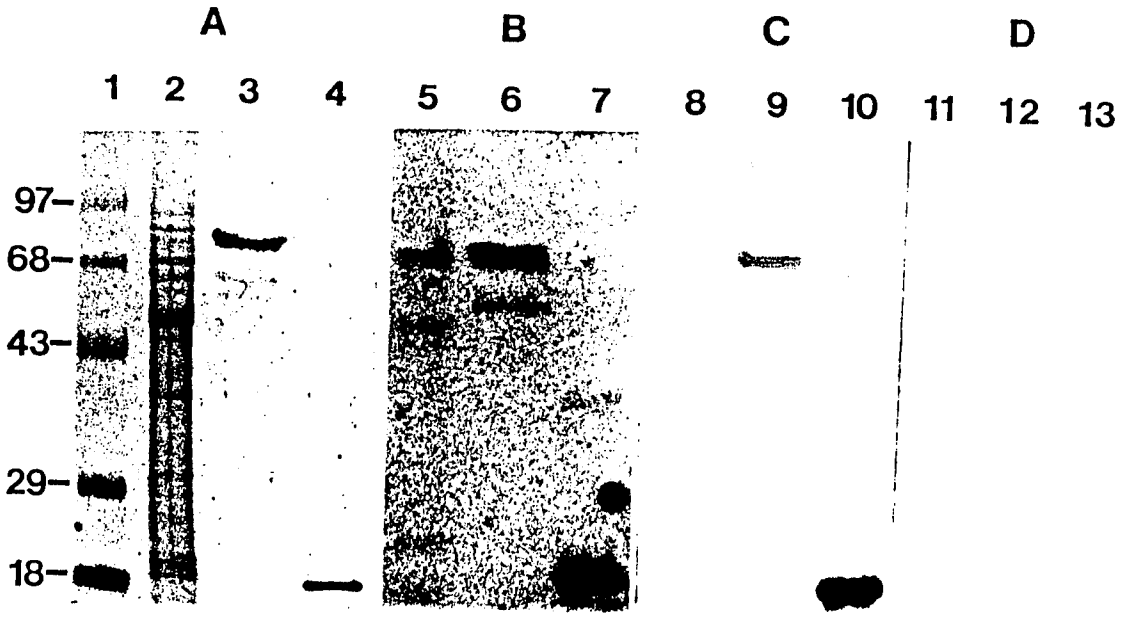

A
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14
1 2 3 4 5 6 7 8 9 10 11 12 13 14
23.1–
9.4–
6.6–
4.4–
2.3–
2.0–
0.6–


Lt-1r
5'
3'
1.2 Kb
1.0 Kb
1.1 Kb
Lt-1
5'
3'
99 bp repeats
0.7 Kb
0.7 Kb
Lt-2
5'
3'
60/99 bp repeats
0.2 Kb
1.5 Kb
1 Kb
Lt-3
5'
3'
24 bp repeats


0.40
0.30
0.20
0.10
0.00
absorbance (405 nm)
Lysate
rLt-1
VTL-C
VTL-S
GWS
RC-N
A-N


absorbance (405 nm)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Lysate
rLt-1r
VTL-C
VTL-S
GWS
RC-N
A-N


absorbance (405 nm)
0.50
0.40
0.30
0.20
0.10
0.00
Lysate
rLt-2
VTL-C
VTL-S
GWS
RC-N
A-N


A
B
C
D
1 2 3 4 5 6 7 8 9 10 11 12 13
97–
68–
43–
29–
18–

F.4.j.

Publications:

Dillon, D.C., C.H. Day, and S.G. Reed. Cloning of Leishmania tropica antigen genes with potential serodiagnostic utility. (in preparation).

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